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ORIGINAL PAPER

Effect of polyvinyl alcohol on in vitro rooting capacity of shoots in pear clones (*Pyrus communis* L.) of different ploidy

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Abstract Poor adventitious root formation is a major obstacle in micropropagation. In this study, intense efforts have been made for improvement of rooting procedures for triploid, tetraploid and mixploid clones of the pear cultivar 'Fertility' obtained by in vitro colchicine treatment. An efficient rooting procedure has been developed for the pear clones of different ploidy by adding polyvinyl alcohol (PVA) to the rooting medium. PVA significantly improved rooting percentage and root number per plantlet for diploid, triploid and tetraploid clones. However, PVA had little or no effect on rooting capacity of mixploid clones. The efficiency of in vitro rooting in diploid, triploid, tetraploid and mixploid is highly genotype-dependent. In general, diploid was the highest, followed by triploid and tetraploid, mixploid was the lowest.

Keywords *Pyrus communis* · Polyploid clones · Polyvinyl alcohol · In vitro shoots · Rooting

Abbreviations

PVA	Polyvinyl alcohol
IBA	Indole-3-butyric acid
BA	6-Benzyladenine
MS	Murashige and Skoog
QL	Quoirin and Lepoivre

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Introduction

Efficient in vitro rooting of shoots is a critical step in micropropagation of fruit crops. Losses at this step will have vast economic consequences. The ability of in vitro shoots to form roots is affected by several factors, including differences between genotypes (Bertazza et al. 1995; Barros et al. 2005), culture procedures (Anirudh and Kanwar 2008; Bertazza et al. 1995; Barros et al. 2005; Dolcet-Sanjuan et al. 1990), mineral nutrition (Liu et al. 2004; Ramage and Williams 2002), subculture times (Banno et al. 1989; Tang et al. 2006), the level of tissue maturity (Olivier 2004), and physiological age (Tang et al. 2006). Due to these factors, in vitro shoots showing a variety of rooting responses have resulted in plants being variously described as easy-to-root (ETR), or difficult-to-root (DTR) (Marks and Simpson 2000). These terms define how well a shoot roots on its own, or in response to exogenous auxin (Geneve 1993). In this paper, the in vitro rooting capacity of polyploids originated from the diploid cultivar 'Fertility' showed different responses to exogenous auxins in our earlier observations, with the diploid control clone being easy to root and the polyploid and mixploid clones being difficult to root. Rooting methods for other *Pyrus* species that had been published (Bertazza et al. 1995; Barros et al. 2005; Dolcet-Sanjuan et al. 1990; Liu et al. 2004; Luo et al. 2006; Marino 1988; Marks and Simpson 2000; Shibli et al. 1997) were not effective for the polyploid clones in our preliminary experiments. It had been reported that PVA had been used for seed coating or pretreatment seed to improve seed germination, seedling growth or resistance to salt (Lin et al. 2003; Guo et al. 1989; Hong and Zhao 1997; Ruan and Xue 2002; Xiong 1998), to increase chlorophyll content in peas (Czeczuga and Nowak 1962), or used in soil to reduce runoff and soil losses (Marsh and Groenevelt

1992) and to stabilize soil (Stefanson 1973; Oades 1976). Recently there have been reports that PVA could be used to reduce vitrification in plant tissue culture, improve root number and root length in jujube (Wang et al. 2006) and improve rooting percentage in woad (Zhang et al. 2007). But the effect of PVA on rooting capacity of DTR polyploid pear clones have not been investigated. In this paper, we first report that PVA efficiently improves in vitro rooting capacity of shoots of DTR polyploid pear clones.

Materials and methods

Plant material and culture condition

In vitro shoots of the diploid pear cultivar 'Fertility' (*Pyrus communis* L.) (2×) and three triploid (3×-1, 3×-2, 3×-3), three tetraploid (4×-1, 4×-2, 4×-3) and three mixploid (Mix-1, Mix-2, Mix-3) clones (ploidy levels were determined by flow cytometry) derived from it by in vitro colchicine treatment were proliferated and maintained on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with 1 mg l^{-1} BA and 0.2 mg l^{-1} IBA, 3% sucrose and 0.6% agar, and with the pH adjusted to 5.8 before autoclaving (121°C, 20 min). The cultures were grown at 25°C with a photoperiod of 16-h light (40 μmol m^{-2} s^{-1}, as provided by cool-white fluorescent light) and 8-h darkness.

Root induction

In vitro shoots, 1.5 cm or longer, were excised from all pear clones were transferred onto root induction medium (RM) containing 0.5 mg l^{-1} IBA and 2% sucrose. The choice of these 0.5 mg l^{-1} IBA and 2% sucrose was based on an earlier experiment (data not shown) that these concentrations were effective for root formation of the diploid pear shoots. In the first experiment the effects of three basal media, 1/2-strength MS (1/2MS); 1/4-strength MS (1/4MS) and 1/2-strength QL (Quoirin and Lepoivre 1977) (1/2QL) were tested. In the second experiment 1/2QL was selected as the basal medium, which was supplemented with 0.5 mg l^{-1} IBA. The effects of various concentrations of PVA (0.0, 0.5, 1.0, 1.5 and 2.0 g l^{-1}) were examined. Because PVA was stable under autoclaving conditions (Koziarz and Yamazaki 1999), PVA was added before medium was autoclaved. The diploid and only two triploid, two tetraploid, and mixploid clones were used in the second experiment. The percentage of rooted shoots and the number of roots per plantlet were recorded for each treatment after 6 weeks.

Plantlets were gently removed from the container and thoroughly washed with tap water to remove adherent medium, then planted in a small pot (the size: 10 × 8 cm, h × d) with peat, perlite, and vermiculite in 1:1:1 (v/v/v) ratio in greenhouse. After 80 days, the number of surviving plants was counted and the survivors were transferred into the field.

Statistical analysis

In both experiments, each treatment included five jars (the size: 10 × 6 cm, h × d), with each jar containing ten shoots, for a total of 50 shoots as one replicate. The experiments were performed 3 times. The jars for each replicate were arranged in a completely randomized design.

Means for each medium-clone-replicate were computed and analyzed. Data on the effect of medium on percentage of shoots rooting were analyzed according to a randomized complete block design with each replicate being a block. The program SAS PROC MIXED (Littell et al. 1996) was used for the analysis according to a mixed effects model. Medium and clone were treated as fixed effects and replicate and the interactions with medium and clone were treated as random effects. Differences among media within each clone were analyzed using the SLICE option and mean separation was performed using the lsd ($P = 0.05$). A separate analysis within each clone was also performed to confirm the results of the overall analysis.

Means for each clone-PVA concentration-replicate were computed and analyzed, so no transformations were performed. Data on the effect of PVA on percentage of shoots rooting and number of roots per shoot were analyzed according to a randomized complete block design with each replicated being a block and with PVA concentration as a covariate (i.e. quantitative factor). The linear model included linear and quadratic effects of PVA concentration. The program SAS PROC MIXED (Littell et al. 1996) was used for analysis according to a mixed effects model with clone treated as s fixed effect and replicate and the interactions treated as a random effect.

Results and discussion

Effect of basal medium on rooting percentage of pear clones of different ploidy

The main effects of medium and clone were statistically significant ($Pr > F < 0.0001$). The effect of the medium by clone interaction was also significant ($Pr > F < 0.0001$), probably due to the general lack of rooting on 1/2 MS and the lack of rooting by clone Mix-3. Significantly higher rooting percentage was obtained on 1/2 QL as compared to 1/2 MS and 1/4 MS (Table 1) except for 3×-1

Table 1 Effect of basal media on rooting percentage in different ploidy pear clones

Basal media*	Rooting percentage %									
	2×	3×-1	3×-2	3×-3	4×-1	4×-2	4×-3	Mix-1	Mix-2	Mix-3
1/2MS	15.2 ± 2.4c	0c	0c	0c	0c	0c	0c	0c	0c	0
1/4MS	31.2 ± 2.2b	23.5 ± 3.2a	13.2 ± 2.3b	18.7 ± 2.7b	9.7 ± 0.8b	12.6 ± 1.5b	8.9 ± 1.1b	15.6 ± 2.4b	9.9 ± 1.9b	0
1/2QL	70.0 ± 4.0a	21.0 ± 2.1a	50.5 ± 5.1a	53.6 ± 3.1a	24.5 ± 1.8a	35.0 ± 3.0a	33.3 ± 2.5a	20.3 ± 2.5a	17.8 ± 2.3a	0

Mean values ±SE followed by a different letter in each column were significantly different at $P < 0.05$ by LSD test

* Each basal medium with 0.5 mg l^{-1} IBA and 2% sucrose

and Mix-3. There was no significantly difference between 1/4 MS and 1/2 QL for 3×-1 and no root formation at any media for Mix-3. The result was in agreement with the finding of Cosac et al. (2008) that 1/2 MS only induced rooting of the easy-to-root cultivar 'Conference', but failed to induce rooting of difficult-to-root cultivars. Rooting percentages on 1/2 QL varied among ploidy levels, with diploid being the highest (70%), followed by triploids (41.7%), tetraploids (30.9%) and then mixploids (12.7%). The low rooting percentage affected the efficiency of transplantation of the polyploid clones and evaluation in the field. It was also found that 1/4 MS resulted in more callus at the bases of shoots (Fig. 1A, B, C) and the adventitious roots (Fig. 1C, D). The finding of low rooting percentage on 1/4 MS is in conformity with the findings of Anirudh and Kanwar (2008) that a high degree of basal callusing resulted in poor rooting response in wild pear. Callus formation has also been found to adversely affect transplant survival rate (Banno et al. 1988; Banno et al. 1989, Stefancic et al. 2005). In order to improve the rooting percentage and rooting quality of polyploids, 1/2 QL was selected as basal medium in the following experiment to test the effect of PVA on rooting capacity. Half-strength QL medium was best medium to induce in vitro rooting, possibly due to higher ionic concentration of NO_3/NH_4 in 1/2 QL than in 1/2 MS and 1/4 MS (data not shown), as previously reported (Quitério et al. 2008) that higher ionic concentration of NO_3/NH_4 increased rooting frequency of pear rootstock and root number and root length were linearly decreased with increasing NH_4/NO_3 ratio in *Phalaenopsis* seedlings (Kubota et al. 2000).

Effect of PVA on in vitro rooting capacity of shoots of pear clones of different ploidy

The analysis of covariance indicated that there were significant differences among clonal means ($Pr > F < 0.0001$), and that the interaction of PVA and clone was also significant ($Pr > F = 0.002$). The linear and quadratic effects of PVA were also significant ($Pr > F < 0.0001$), as was the interaction of the quadratic PVA and clone ($Pr > F = 0.004$) effect. Thus, the response curves for each clone differed (Fig. 2). PVA treatment had a significant positive effect on rooting percentage for all clones (Fig. 2A), exclusive of Mix-3. The rooting percentages of all clones obtained with PVA and without PVA were

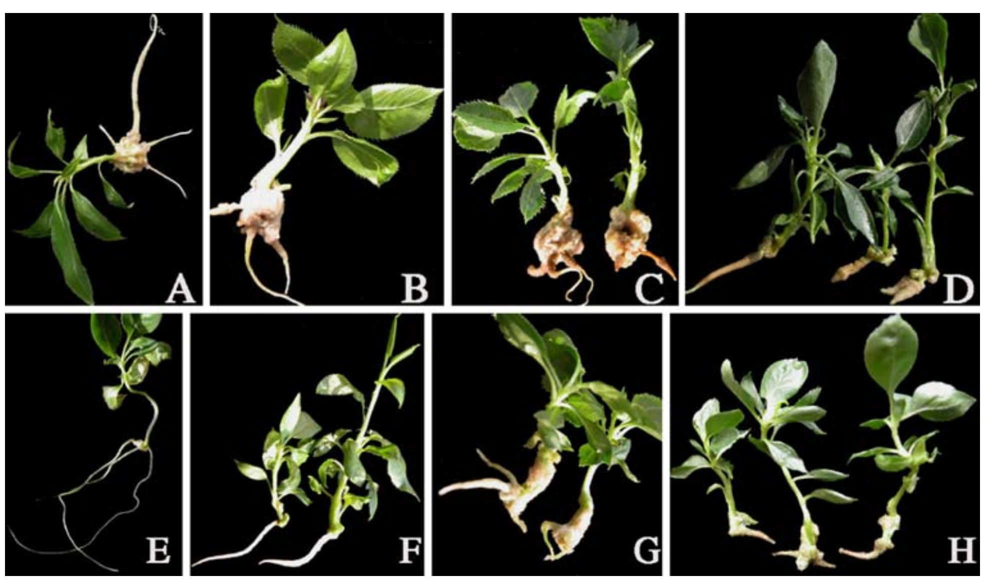

Fig. 1 The effect of basal medium on in vitro rooting of shoots in different ploidy pear clones. **A**, **B**, **C**, **D**: 1/4 MS medium; **E**, **F**, **G**, **H**: 1/2QL medium. **A**, **E**: diploid; **B**, **F**: triploid; **C**, **G**: tetraploid; **D**, **H**: mixploid

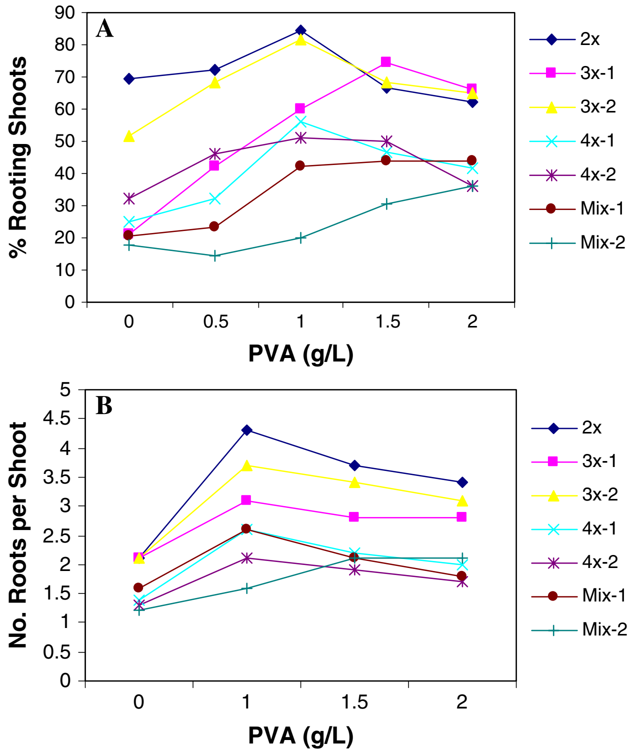

Fig. 2 Effect of PVA on rooting (**A**) and root number per plantlet (**B**)

significantly different. But the optimal PVA concentration was different for clones of different ploidy levels. For the diploid (2×), the rooting percentage at 1.0 g l^{-1} PVA was the highest. For 3×-1, the rooting percentage was the highest at 1.5 g l^{-1} PVA. For 3×-2 and both 4×-1 and 4×-2 the highest rooting were observed at 1.0 g l^{-1} PVA. The rooting percentage of Mix-1 was the highest at 1.5 g l^{-1} PVA, and that for Mix-2 was the highest at 2.0 g l^{-1} PVA. Mix-3 had no root formation at any PVA concentration. Thus, there were no large differences in optimum concentration among the clones with the exception of mixiploid clones requiring somewhat higher concentrations. However, percentage of rooting was higher in the diploid clone and 3×-1 and 3×-2 triploid clones.

The analysis of covariance indicated that difference among clonal means were significant ($Pr > F = 0.001$). The linear and quadratic effects of PVA concentration were also significant ($Pr > F = 0.0001$). The interaction of the linear and quadratic effects of PVA concentration with clone were also significant ($Pr > F = 0.014$ and 0.028, respectively), indicating that the response curves for the clones differed (Fig. 2). PVA treatment significantly improved root number per plantlet in pear clones of different ploidy levels (Figs. 2B, 3A, B, C, D). The highest root numbers were observed at 1 g l^{-1} PVA for all clones exclusive of Mix-2, which had the greatest response at 1.5 or 2.0 g l^{-1} PVA. Root number per plantlet was different between different ploidy levels. In general, the diploid clone was highest, the triploid clones were second, and the tetraploid and mixploid clones were lowest. It was also found that PVA treatment improved root thickness in the diploid clone (Fig. 3A), similar to a previous report for jujube (Wang et al. 2006), but PVA had no evident effect on root thickness of the polyploid clones.

Stefanson (1973) reported that PVA application in soil increased wheat seeds emergence rate due to PVA responsible for a degree of stabilization of the soil surface. Mikkelsen (1994) reported that fertilizer-containing solutions were mixed with hydrophilic polymers to form a gel, the release of soluble nutrients can be substantially delayed compared with soluble fertilizer alone, so that plant recovery can be increased because of reducing nutrients leaching. In this study, nutrient-containing rooting medium is mixed with synthetic polymer PVA to make a new rooting medium. This new medium containing an appropriate concentration of PVA improved in vitro rooting capacity of shoots of pear clones of different ploidy, though information regarding the exact interaction of nutrients and PVA was not clear. The action mechanism of PVA and nutrients in medium is worthy of further study.

Survival of plants in transplantation

Plantlets were transferred into pots in the greenhouse. The survival frequency, similar to the rooting percentage, varied among ploidy levels, with diploid being the highest (90%), followed by triploids (70%), tetraploids (60%), and then mixploids (45%). The survival frequency of plants from pots to the field was 100% for all ploidy levels (Fig. 4). Low survival rate of plants when they are

Fig. 3 The effect of 1/2QL with PVA on in vitro rooting of shoots in different ploidy pear clones. **A**: diploid; **B**: triploid; **C**: tetraploid; **D**: mixploid

Fig. 4 Plantlets of different ploidy levels grow in pots and in the field

removed from in vitro culture was associated with poor stomatal functioning and excessive water loss (Brainerd and Fuchigami 1982). Sutter and Langhans (1982) reported that because cabbage plants in vitro had no structured epicuticular wax whereas plants grown in a growth chamber or in the greenhouse had considerable amounts of structured epicuticular wax, water loss per unit leaf area was greater in plants removed from in vitro conditions than in plants grown in the growth chamber, transplant survival rate of cabbage was low. In this paper, the reason of low surival rate of tetraploids and mixploids in external conditions was not fully known.

In conclusion, in vitro rooting capacity was decreased with the increase of ploidy in the same variety, with diploid being easy-to-root and mixploids being difficult-to-root. Supplementation of PVA in the rooting medium increased rooting percentage and root number per plantlet of polyploid clones. This demonstrated that PVA can be used as an additive to improve rooting capacity of low rooting capacity pear clones, but can not be used to induce root formation of unrooting pear clones such as the mixploid clone Mix-3. The effect of PVA on in vitro rooting capacity may be applicable to the improvement of other difficult-to-root pear or other fruit crops.

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